School of Biomedical Engineering, Science and Health Systems

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Improving the efficacy of cellular therapy by magnetic cell targeting

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Background

Objectives

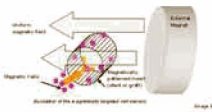
- To extend our concurrent efforts in magnetic drug delivery to coronary stents using magnetic nanoparticles to the delivery of cells and biological agents
- To accomplish this ***in vivo*** by applying high magnetic field gradients within the body to an injected suspension of magnetized cells, without relying on large external magnetic gradients to apply forces

Why Target Cells with Magnets?

- **Utilizes our effective drug targeting technique**
- **Capabilities:**
  - Efficient localization of cells
  - Manage small resource supply
  - Minimally invasive
  - Improve quality of treatment
  - Autologous or Non-immunogenic Cell Source
- **Applications:**
  - Implant endothelialization
  - Vascular tissue regeneration
  - Cells as a drug delivery vehicle

High Gradient Magnetic Delivery

- The Magnetic Implant-Based Cell & Drug Delivery System
  - Weakly magnetize stents, or impregnate scaffolds/grafts with nanoformulations of magnetite
  - Inject magnetically loaded cells with the aid of a modest external field, allowing highly efficient targeting of cells or drugs
  - The magnetizable features of the implant, when exposed to a uniform magnetic field, can apply the forces needed to capture susceptible agents from blood flow



Our method: Two magnetic sources

1. One source: Maximize the carrier's moment $\vec{m} = \chi \vec{V} \vec{H}$

2. Another source: Maximize the field gradient $\nabla \vec{H}$

$$\vec{F} = \mu_o (\vec{m} \cdot \nabla) \vec{H}$$

Independently controlling these variables can optimize the forces which can be applied on injected drug carriers

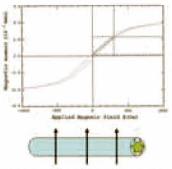
Experiments

Cell Culture Models

- Objectives
  1. Develop a reproducible method for quantifiable loading of endothelial cells with superparamagnetic particles
  2. Particle uptake in suspension or adherent culture, and subsequent biological response.
  3. Magnetic capture of endothelial cells with internalized nanoparticles in parallel plate flow and in vivo

Model Implant: *Wire Mesh*

Magnetic Measurements



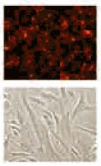
Determination of a 0.05 Tesla saturation for the applied magnetic field by Alternating Gradient Magnetometer measurement of a single magnetically plated wire

Cell Loading Groups

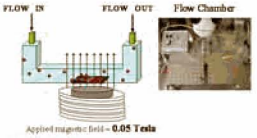
- Bovine Aortic Endothelial Cells
  - 350nm diameter, rhodamin-stained polystyrene magnetic particles, carboxyl terminus
  - Preliminary non-degradable particle model
- Human Umbilical Vein Endothelial Cells
  - 130nm diameter, dextran magnetic particles, NH2 terminus
  - Degradable particles suitable for in vivo applications

Particle loading to ECs

- Particle suspension vortexed
- Particles seeded to adherent cultures in BAECS, in suspension to HUVECs
- No magnetic field source used
- Cells with endocytosed particles
  - Magnetometer analysis concludes approximately 10^7 particles per BAEC at saturation
  - No saturation data for 130nm particles, but controllably load at ~10^5 particles per cell



In Vitro Experimental Setup



Applied magnetic field – **0.05 Tesla**

Flow velocity – **15 cm/s**

- Magnetically electroplated "unrolled" steel mounted on channel floor
- Steel is cured in nitrate and collagen coated to simulate tissue integration

Current In Vivo Studies (Unloaded Magnetic Particles)

- Sprague Dawley Rats – non survival
- 130nm magnetic particles
  - Dextran encapsulated
  - Tc99m linked to PEG-NH$_2$
- 1mm diameter, 5mm long stainless steel stent implanted in common iliac artery
- Deliver dose intra-venously under 0.05 Tesla applied uniform magnetic field for varied time steps
- Harvest device, organs, for biodistribution counts

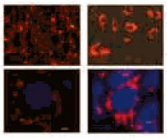
Surgery Images

A rat receives a magnetic particle dose intravenously under exposure to a uniform static magnetic field at 0.05 Tesla

Results

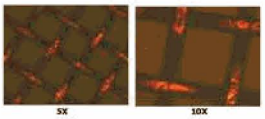
In vitro Loading Studies and Parallel Plate Flow Chamber Analysis

Magnetic Endothelial Cells



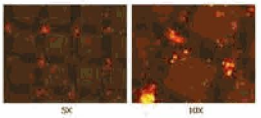
(TOP) Fluorescent images of Bovine Aortic Endothelial Cells loaded with ~10^4 350nm diameter magnetic particles. (BOTTOM) Confocal images of DAPI stained BAECs loaded with 350 nm magnetic particles

Capture Results – 30 minutes



Dense capture of magnetically loaded BAECs is seen along the wires of the magnetic stent, with virtually no cells in between the struts.

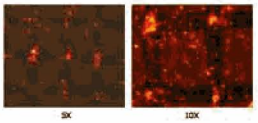
Capture Results – 6 Hours



After 24 hours in culture at 37°C 5% CO$_2$

Magnetically loaded BAECs are beginning to migrate off the intersections and attach elsewhere

Capture Results – 24 hours



After 24 hours in culture at 37°C 5% CO$_2$

At 24 hours, MSAECs have continued to migrate and divide across the substrate, indicating the ability of mature cells loaded with magnetic particles to withstand the trauma of applied magnetic forces

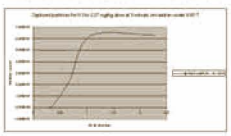
In Vivo Magnetic Particle Delivery to Stents in a Rat Common Iliac Artery

Qualitative Results

X-ray of implanted stent (contrast added) and gamma image of biodistribution.

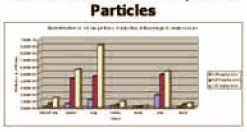
The gamma images clearly indicate the high activity at the stent implant site (lilac artery). All views are of the same animal, and the liver was removed for clarity.

Dose Dependent Delivery



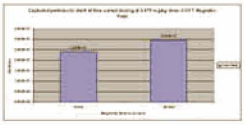
Dose dependent capture was seen over a range of doses, beginning to level off at 1 mg/kg. This data reflects only one field strength and exposure time.

Biodistribution of Uncaptured Particles



Consistent relationships in biodistribution shown, encouraging in the capability to reduce uptake in the lungs and liver while maintaining robust stent capture

Time Varied Magnetic Dosing



A 25% increase in particle capture was seen over a 15 minute increase in field exposure before sacrifice and organ harvest

Conclusions

Proof of Principle

- Functionality of the our method
  - ✓ Sub-micron and nano-scale magnetic particles can be captured, by controllable means, to the surface of a magnetizable implant
- Flexibility in application
  1. A microlayer of soft magnetic deposition on a stent or other non-vascular implants
  2. Varying the alloy content of a stent or other non-vascular implants
  3. Impregnating magnetite crystals into a polymer scaffold or woven graft

Transitions to Cellular Therapy

- Biocompatibility
  - ✓Vascular endothelial cells have been shown to grow upon magnetized surfaces, uptake magnetic nanoparticles, maintaining normal growth rates, morphology, and behavior.
  - ✓These endothelial cells can be delivered magnetically to the surface of a stent and withstand potentially traumatic forces

Implementation

From particles, to mature cells, to stem cells

1. As seen recently with lab-grown bladders comprised of mature cells, in many cases it is not necessary to involve stem cells when adequate autologous sources of mature cells are available
2. This project is following a logical path of adapting to mature vascular endothelial cells, while beginning to explore effects on differentiation in cord blood stem cells loaded with magnetic nanoparticles.
3. As we have demonstrated that cells can be loaded in suspension in controlled fashion, in the clinic this may limit procedural steps and risks of contamination
4. Endothelial cell suspensions loaded with nanoparticles will comprise a magnetic susceptibility factors of 10 ahead of individual particles, already shown to be accurately targeted on their own in vivo, foreshadowing further reduced amounts of magnetic material needed, and in turn a more efficient method of treatment

Future Work

- NZW Rabbit in vivo model
  - Pharmacokinetics of magnetic ECs
  - Toxicity / Risk
  - Tissue integration
- Product prototyping (magnetic stent, magnetic collagen scaffold)
- Expanding applications
  - Cord blood stem cells
  - Magnetic bioabsorbable staples, sutures
  - Wound care
  - Your idea here!